

Revealing wild Cannabis at the territory of North Macedonia

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Introduction

Scientific interest in cannabis (*Cannabis sativa* L.) has grown exponentially in the last 30 years due to the discovery of beneficial phytochemicals along with the study of the human endocannabinoid system. Accordingly, changes in legislation were initiated to legalize the use of cannabis for medical purposes. Anthropogenic pressure on this plant arises due to its use value which is directly related to its phytochemical composition. More than 545 metabolic components have been identified by Cannabis, about 144 of which as phytocannabinoids present as acidic forms. The most important acidic forms of phytocannabinoids are tetrahydrocannabinolic and cannabidiolic acid (Δ^9 -THCA and CBDA). Dried cannabis flower is the official raw material and pharmacopoeial monographs recommend determination of the content of five cannabinoids (CBD, CBDA, CBN, Δ^9 -THC and Δ^9 -THCA) and differentiation between hemotypes (Stefkov et al., 2021). Cannabis is cultivated as an important industrial or medicinal plant, but can also be found as a wild species in Republic of North Macedonia. The purpose of the study is to investigate whether there is wild growing Cannabis in North Macedonia through localization of the areas where it grows, collection of plant material, morphological and phytochemical characterization and also to assess its commercial value.

Materials and methods

Plant material

Plant material was collected from 5 localities with wild growing Cannabis plant (Kochansko region, Republic

of North Macedonia: L1 - Zletovo river (July 2019, August 2020), L2 - Mojanci (July 2019), L3 - Banja Trkanje (July 2019), L4 - between Gorni and Dolni Podlog (July 2019) and L5 - Dolni Balvan (August 2020). The collected plant material comprises herbarium specimen, leaves and seeds. Cannabis specimens were identified by Prof. Dr. Gjose Stefkov Institute for Pharmacognosy, herbarized, stored and voucher specimens (FFUKIMCs(1,4,8,9/19), FFUKIMCs(1/20, 2/20) at the Herbarium at the Faculty of Pharmacy, UKIM.

Reagents

Cannabidiol CRM solution, cannabinol CRM solution, (-)- Δ^9 -tetrahydrocannabinol CRM solution, Δ^9 -tetrahydrocannabinolic acid A CRM solution and cannabidiolic acid CRM solution with concentration of 1 mg/mL were purchased from Cerilliant Corporation (USA). 85% o-phosphoric acid and acetonitrile HPLC grade were purchased from Carlo Erba. Ethanol 96% Ph.Eur. grade was purchased from Alkaloid AD Skopje.

Morphological characterization

Cannabis plants were characterized using UPOV (The International Union for the Protection of New Varieties of Plants) descriptors. For that purpose, plant material (leaves and seeds) were collected for further analysis (International Union for the protection of the new varieties of plants Geneva, 2012).

HPLC characterisation of phytocannabinoids

For determination of cannabinoid content in collected Cannabis leaves as plant material, DAB Pharmacopoeial method for assay of cannabinoids was applied. The chromatographic analyses were carried out using Agilent 1200 Model HPLC equipped with DAD G1315D, quaternary pump G1311A, column thermostat G1316A and thermostatted autosampler G1329A (Agilent Technologies, USA). Separation was achieved using InfinityLab Poroshell 120 EC-C18 chromatographic column (150 mm x 3 mm ID, 2.7 µm, Agilent Technologies, USA) at 40 °C with mobile phase consisting of aqueous solution of o-phosphoric acid (8.64 g/L) as solvent A and acetonitrile as solvent B. Sample preparation (triplicates) and loss on drying for each sample was determined according to DAB monograph for Cannabis flower (German Pharmacopoeia 2020th ed. 2020).

Results and discussion

For general information on the localities of wild growing Cannabis plants on territory of North Macedonia, ethnopharmacological survey was conducted. Six localities were pointed out and at five out of six Cannabis plants were identified during terrain activities.

A number of morphological analyses were made on plant material (leaves and seeds) using nine UPOV descriptors (D) for leaves and three for seeds. The analyses revealed the following results (average values of all five locations): D6 (descriptor 6) - leaf length of petiole were approximately 30.15 mm and 5.59 mm on lower leaves and upper leaves, respectively; D7 - leaf: anthocyanin coloration of petiole was weak; D8 - number of leaflets was 7 or 9 on lower leaves and 3 or 5 on upper leaves; D9 - central leaflet length was average 80.10 mm on lower leaves and 51.32 mm on upper leaves; D10 - central leaflet width with average value 11.65 mm on lower leaves and 2.48 mm on upper leaves; D19 - main stem length of internode, approximately 139.9 mm; D20 - main stem thickness characterized 9.47 mm; D21 - main stem depth of grooves was shallow; D22 - main stem: pith in cross-section was medium. Next three descriptors are for seed characterisation, D23 - seed 100 weight was 0.0084415g; D24 - seed color of testa was gray brown to yellowish brown and D25 - seed: marbling was strong.

HPLC phytochemical analysis of dry leaves has shown that CBDA was predominant component in all of the samples, ranging 0.3 - 1.41% m/m. CBD content varied from 0.01 - 0.18% m/m, while THC complied to the official limit for THC in hemp and hemp products (below 0.02% m/m) except for location L3 (0.03%). THCA was below limit of quantification and CBN was not detected in any of the samples. Highest CBDA content was noted in sample

L3 (1.41%), following L1 from August 2020 (1.37%, 1.12%, 0.68%) and L1 July 2019 (0.46%), L4 from 2019 (0.43%, 0.35%), L2 from 2019 (0.40%) and L5 from 2020 (0.36%). According results from L1 from July 2019 and August 2020 seems that technical maturity and better harvest period is probably at August.

Conclusion

The morphological characterization confirmed that all collected samples are same phenotype *C. sativa* spp. *sativa*. The phytochemical characterization analysis from 5 localities of central eastern Macedonia, in Kocani, Shtip, Probistip confirmed predominant presence of total CBD up to 1.42% m/m and total THC below 0.02% m/m (with exception of 0.03% in L3 – Banja Trkanje).

As a genetic resource, regardless of the low content of CBD and THC, wild cannabis species have the potential to be one of the parents in future hybridization and obtaining new hybrid varieties that could be successfully cultivated outdoors in North Macedonia. By hybridization with other, more potent varieties of Cannabis, they can be enriched with desired content of cannabinoids.

These results will be properly disseminated and will contribute to the valorization of this plant, raise awareness of the existence and importance of indigenous Cannabis in North Macedonia and how to change the practice of its intentional destruction to preservation.

References

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